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Structural Studies of Protein Prenyltransferases

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Beamline(s): X12B

Introduction: Protein geranylgeranyltransferase type I (GGTase-I) and protein farnesyltransferase (FTase) are essential enzymes involved in the post-translational modification of many critical cell signaling proteins; including Ras, RhoB, Rac, and most heterotrimeric G protein gamma subunits. These enzymes catalyze the covalent addition of either a 20 carbon geranylgeranyl isoprenoid or a 15 carbon farnesyl isoprenoid to the C-terminus of target proteins. Mutations in Ras are associate with over 30% of all human cancers and isoprenoid attachment is required for proper function of Ras, where it promotes membrane association and mediates certain protein-protein interactions. This requirement has provided promising targets for therapeutic agents designed to interrupt cell signaling pathways and several inhibitors of FTase are now in clinical trials for the treatment of cancer.

Methods and Materials: Standard methods for macromolecular crystallography at cryogenic temperatures were employed for collection of the diffraction data. Phases for the initial GGTase-I structure were determined using both the isomorphous and anomalous scattering differences from a single mercury derivative (SIRAS). Substrate complexes were created by co-crystallization, or in some cases substrates were added by soaking a crystal in a solution containing the substrate and allowing it to diffuse through the crystal lattice.

Results: The structure of mammalian GGTase-I has been determined using diffraction data collected at beamline X12B, providing the first look at this important enzyme (Fig. 1). Additional structures determined at X12B include GGTase-I with two different peptide substrates derived from K-Ras4B, GGTase-I with a prenylated peptide product, and a GGTase-I substrate complex in which manganese was substituted for magnesium. In concert, these structures detail aspects of the GGTase-I reaction mechanism; including substrate selection, the role of metal ions, and product binding.

Structural studies of the FTase enzyme have also continued with the collection of data from complexes of FTase with peptides derived from H-Ras and Rap2a proteins, a product complex, and several different FTase specific inhibitors designed as potential drug candidates.

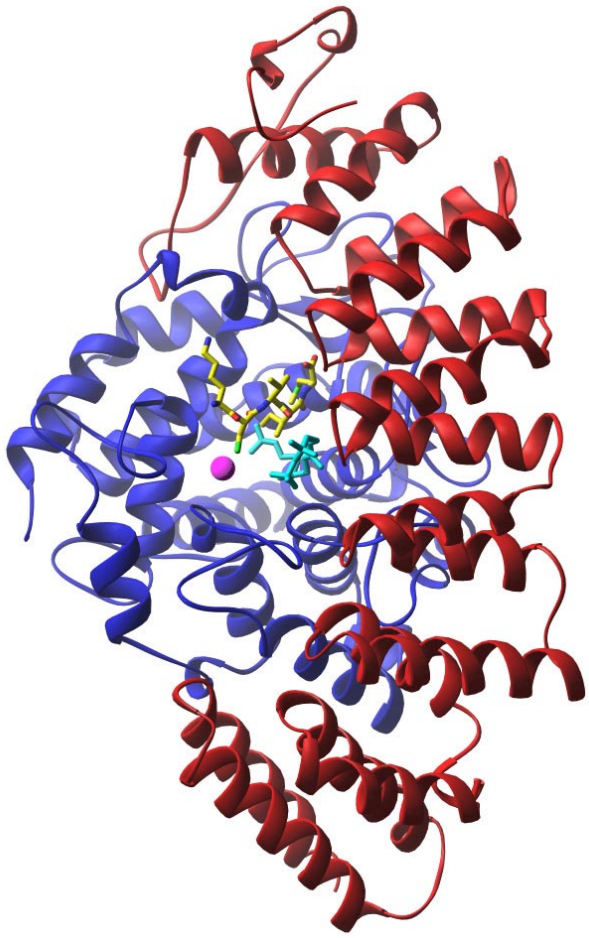

Figure 1. Geranylgeranyltransferase Type-I ternary complex. GGTase-I is a heterodimer composed of a 48KDa alpha subunit (*red*) and a 43KDa beta subunit (*blue*). GGTase-I is a metalloenzyme containing a single zinc ion (*purple*) that coordinates the binding of the substrate peptide (*yellow*). A non-reactive analog of the natural isoprenoid substrate is also bound in the active site (*cyan*).